

INTERNATIONAL SEARCH REPORT

Internal 1 Application No
PCT/EP 94/01625

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C12N15/31 C12N9/80 C12Q1/68 C12P21/08 A61K39/106
G01N33/577

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C12N C12Q C12P A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GASTROENTEROLOGY, vol.104, no.4, April 1993, ELSEVIER, NEW YORK, U.S.;	10,11,20
Y	page A699 R.L. FERRERO ET AL. 'Molecular evidence demonstrating significant homology between the urease polypeptides of Helicobacter felis and Helicobacter pylori' Digestive disease week and the 94th annual meeting of the american gastroenterological association, May 15-21, 1993; Boston, Massachusetts, US; * page A699, left column, paragraph 2 *	7-9, 12-16, 22, 24-28, 31-35

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

10 October 1994

Date of mailing of the international search report

27. 10. 94

Name and mailing address of the ISA

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Hornig, H

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PCT/EP 94/01625

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,93 07273 (INSTITUT PASTEUR) 15 April 1993 cited in the application * the whole document *	7-16, 22, 24-28, 31-35
X	INFECTION AND IMMUNITY, vol.60, no.5, May 1992, AM. SOC. MICROBIOL., BALTIMORE, US; pages 1946 - 1951 B.E. DUNN ET AL. 'Identification and purification of a cpn60 heat shock protein homolog from Helicobacter pylori' cited in the application the whole document	17
X	INFECTION AND IMMUNITY, vol.60, no.5, May 1992, AM. SOC. MICROBIOL., BALTIMORE, US; pages 2125 - 2127 D.J. EVANS ET AL. 'Urease-associated heat shock protein of Helicobacter pylori' cited in the application the whole document	17
Y	WO,A,90 04030 (INSTITUT PASTEUR) 19 April 1990 the whole document	10-16, 20, 21, 24, 34, 35
Y	WO,A,91 09049 (RESEARCH EXPLOITATION LIMITED) 27 June 1991 the whole document	10-16, 20, 21, 24, 34, 35
Y	J. CLIN. MICROBIOL., vol.30, no.3, March 1992, AM. SOC. MICROBIOL., WASHINGTON, DC, US; pages 739 - 741 P.A. FOXALL ET AL. 'Use of polymerase chain reaction-amplified Helicobacter pylori urease structural genes for differentiation of isolates' the whole document	10-16, 20, 21, 24, 34, 35
P,X	WO,A,94 06474 (GALAGEN INC.) 31 March 1994 the whole document	27-32, 37, 38
P,X	WO,A,93 18150 (BIOCINE-SCLAVO S.P.A.) 16 September 1993	17, 20, 22, 24, 25
P,Y	the whole document	34, 35
-/--		

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Internat 1 Application No
PCT/EP 94/01625

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>MOLEC. MICROBIOL., vol.9, no.2, 14 July 1993, BLACKWELL SCI. PUB., OXFORD, UK; pages 323 - 333 R.L. FERRERO AND A. LABIGNE 'Cloning, expression and sequencing of Helicobacter felis urease genes' see page 324, left column, paragraph 2 - page 326, right column, paragraph 1; figures 1,3</p> <p style="text-align: center;">---</p>	<p>7,8, 10-12,16</p>
P,X	<p>ABSTR. GEN. MEET. AM. SOC. MICROBIOL., vol.93, no.0, 19 May 1993 page 127 S. SUERBAUM AND A. LABIGNE 'Cloning and sequencing of the HSPA and HSPB heat shock protein encoding genes of Helicobacter pylori' 93rd general meeting of the american society for microbiology, Atlanta, Georgia, USA, May 16-20, 1993; abstract no. D-182; see abstract</p> <p style="text-align: center;">-----</p>	<p>17-26</p>

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 94/01625

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9307273	15-04-93	FR-A- 2682122	09-04-93
		CA-A- 2120527	15-04-93
		EP-A- 0610322	17-08-94
WO-A-9004030	19-04-90	FR-A- 2637612	13-04-90
		EP-A- 0367644	09-05-90
		JP-T- 3501928	09-05-91
WO-A-9109049	27-06-91	NONE	
WO-A-9406474	31-03-94	AU-B- 4924893	12-04-94
WO-A-9318150	16-09-93	NONE	

(vi) ORIGINAL SOURCE:

(A) ORGANISM : H. felis

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..591

(D) OTHER INFORMATION: /standard_name= "URE I"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

ATG TTA GGT CTT GTG TTA TTG TAT GTT GCG GTC GTG CTG ATC AGC AAC	48
Met Leu Gly Leu Val Leu Leu Tyr Val Ala Val Val Leu Ile Ser Asn	
1 5 10 15	
GGA GTT AGT GGG CTT GCA AAT GTG GAT GCC AAA AGC AAA GCC ATC ATG	96
Gly Val Ser Gly Leu Ala Asn Val Asp Ala Lys Ser Lys Ala Ile Met	
20 25 30	
AAC TAC TTT GTG GGG GGG GAC TCT CCA TTG TGT GTA ATG TGG TCG CTA	144
Asn Tyr Phe Val Gly Gly Asp Ser Pro Leu Cys Val Met Trp Ser Leu	
35 40 45	
TCA TCT TAT TCC ACT TTC CAC CCC ACC CCC CCT GCA ACT GGT CCA GAA	192
Ser Ser Tyr Ser Thr Phe His Pro Thr Pro Pro Ala Thr Gly Pro Glu	
50 55 60	
GAT GTC GCG CAG GTG TCT CAA CAC CTC ATT AAC TTC TAT GGT CCA GCG	240
Asp Val Ala Gln Val Ser Gln His Leu Ile Asn Phe Tyr Gly Pro Ala	
65 70 75 80	
ACT GGT CTA TTG TTT GGT TTT ACC TAC TTG TAT GCT GCC ATC AAC AAC	288
Thr Gly Leu Leu Phe Gly Phe Thr Tyr Leu Tyr Ala Ala Ile Asn Asn	
85 90 95	
ACT TTC AAT CTC GAT TGG AAA CCC TAT GGC TGG TAT TGC TTG TTT GTA	336
Thr Phe Asn Leu Asp Trp Lys Pro Tyr Gly Trp Tyr Cys Leu Phe Val	
100 105 110	
ACC ATC AAC ACT ATC CCA GCG GCC ATT CTT TCT CAC TAT TCC GAT GCG	384
Thr Ile Asn Thr Ile Pro Ala Ala Ile Leu Ser His Tyr Ser Asp Ala	
115 120 125	
CTT GAT GAT CAC CGC CTC TTA GGA ATC ACT GAG GGC GAT TGG TGG GCT	432
Leu Asp Asp His Arg Leu Leu Gly Ile Thr Glu Gly Asp Trp Trp Ala	
130 135 140	
TTC ATT TGG CTT GCT TGG GGT GTT TTG TGG CTC ACT GGT TGG ATT GAA	480
Phe Ile Trp Leu Ala Trp Gly Val Leu Trp Leu Thr Gly Trp Ile Glu	
145 150 155 160	
TGC GCA CTT GGT AAG AGT CTA GGT AAA TTT GTT CCA TGG CTT GCC ATC	528
Cys Ala Leu Gly Lys Ser Leu Gly Lys Phe Val Pro Trp Leu Ala Ile	
165 170 175	

98

GTC GAG GGC GTG ATC ACC GCT TGG ATT CCT GCT TGG CTA CTC TTT ATC 576
 Val Glu Gly Val Ile Thr Ala Trp Ile Pro Ala Trp Leu Leu Phe Ile
 180 185 190

CAA CAC TGG TCT TGA
 591
 Gln His Trp Ser
 195

(2) INFORMATION FOR SEQ ID NO: 8:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 199 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
 (A) ORGANISM : H. felis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Lys Gly Trp Met Leu Gly Leu Val Leu Leu Tyr Val Ala Val Val Leu
 1 5 10 15

Ile Ser Asn Gly Val Ser Gly Leu Ala Asn Val Asp Ala Lys Ser Lys
 20 25 30

Ala Ile Met Asn Tyr Phe Val Gly Gly Asp Ser Pro Leu Cys Val Met
 35 40 45

Trp Ser Leu Ser Ser Tyr Ser Thr Phe His Pro Thr Pro Pro Ala Thr
 50 55 60

Gly Pro Glu Asp Val Ala Gln Val Ser Gln His Leu Ile Asn Phe Tyr
 65 70 75 80

Gly Pro Ala Thr Gly Leu Leu Phe Gly Phe Thr Tyr Leu Tyr Ala Ala
 85 90 95

Ile Asn Asn Thr Phe Asn Leu Asp Trp Lys Pro Tyr Gly Trp Tyr Cys
 100 105 110

Leu Phe Val Thr Ile Asn Thr Ile Pro Ala Ala Ile Leu Ser His Tyr
 115 120 125

Ser Asp Ala Leu Asp Asp His Arg Leu Leu Gly Ile Thr Glu Gly Asp
 130 135 140

Trp Trp Ala Phe Ile Trp Leu Ala Trp Gly Val Leu Trp Leu Thr Gly
 145 150 155 160

Trp Ile Glu Cys Ala Leu Gly Lys Ser Leu Gly Lys Phe Val Pro Trp
 165 170 175

99

Leu Ala Ile Val Glu Gly Val Ile Thr Ala Trp Ile Pro Ala Trp Leu
180 185 190

Leu Phe Ile Gln His Trp Ser
195

100

CLAIMS

1. Immunogenic composition, capable of inducing antibodies against Helicobacter infection, characterised in that it comprises :

i) at least one sub-unit of a urease structural polypeptide from Helicobacter pylori, or a fragment thereof, said fragment being recognised by antibodies reacting with Helicobacter felis urease, and/or at least one sub-unit of a urease structural polypeptide from Helicobacter felis, or a fragment thereof, said fragment being recognised by antibodies reacting with Helicobacter pylori urease ;

ii) and/or, a Heat Shock protein (HSP), or chaperonin, from Helicobacter, or a fragment of said protein.

2. Immunogenic composition according to claim 1 capable of inducing protective antibodies.

3. Immunogenic composition according to claim 1 characterised in that it includes component (i), which comprises or consists of the Helicobacter felis urease structural polypeptide(s) encoded by the ure A and/or ure B genes of plasmid pILL205 (CNCM I-1355), a polypeptide exhibiting at least 90 % homology with the said polypeptide(s), or a fragment thereof having at least 6 amino-acids and being recognised by antibodies reacting with Helicobacter pylori urease.

4. Immunogenic composition according to claim 1, characterised in that it includes component ii) which is a HSP from Helicobacter pylori, or a fragment thereof.

5. Immunogenic composition according to any of preceding claims characterised in that the HSP is HSP A and/or HSP B encoded by the hsp A and/or hsp B genes respectively, of plasmid pILL689 (CNCM I-1356), or a

polypeptide exhibiting at least 75 % homology with the said HSP's, or a fragment of either or both of these proteins having at least 6 amino-acids.

6. Pharmaceutical composition for use as a vaccine in protecting against Helicobacter infection, particularly against Helicobacter pylori and Helicobacter felis, characterised in that it comprises the immunogenic composition of any of claims 1-5, in combination with physiologically acceptable excipient(s) and possibly adjuvants.

7. Proteinaceous material characterised in that it comprises at least one of the Helicobacter felis polypeptides encoded by the urease gene cluster of the plasmid pILL205 (CNCM I-1355), including the structural and accessory urease polypeptides, or a polypeptide having at least 90 % homology with said polypeptides, or a fragment thereof.

8. Proteinaceous material according to claim 7, characterised in that it consists of or comprises the gene product of ure A and/or ure B as illustrated in figure 3, or a fragment having at least 6 amino-acids, or a variant of these gene products having at least 90 % homology, said fragment and said variant being recognised by antibodies reacting with Helicobacter pylori urease.

9. Proteinaceous material according to claim 7 characterised in that it consists of or comprises the gene product of ure I, as illustrated in figure 9, or a fragment thereof having at least 6 amino-acids, or a variant of the gene product having at least 75 % homology, said fragment and said variant having the capacity to activate the ure A and ure B gene products in the presence of the remaining urease "accessory" gene products.

10. Nucleic acid sequence characterised in that it comprises :

- (i) at least one sequence coding for the proteinaceous material of any one of claims 6-9 ;
- or (ii) a sequence complementary to sequence (i) ;
- or (iii) a sequence capable of hybridising to sequences (i) or (ii) under stringent conditions ;
- iv) a fragment of any of sequences (i), (ii) or (iii) comprising at least 10 consecutive nucleotides.

11. Nucleic acid sequence according to claim 9 characterised in that it comprises the sequence of plasmid pILL205 (CNCM I-1355), for example the sequence of Figure 3, in particular that coding for the gene product of ure A and for ure B or the sequence of Figure 9 (Ure I), or a sequence capable of hybridising to these sequences under stringent conditions, or a sequence complementary to these sequences, or a fragment comprising at least 10 consecutive nucleotides of these sequences.

12. Expression vector characterised in that it contains a nucleic acid sequence according to claim 10 or 11.

13. Plasmid pILL205 (CNCM I-1355).

14. Oligonucleotide suitable for use as a primer in a nucleic acid amplification reaction, characterised in that it comprises from 10 to 100 consecutive nucleotides of the sequence of claim 10 or 11.

15. Nucleotide probe characterised in that it comprises a sequence according to any one of claims 9 or 10, with an appropriate labelling means.

16. Prokaryotic or eukaryotic host cell stably transformed by an expression vector according to claim 12 or 13.

17. Proteinaceous material characterised in that it comprises at least one of the Heat Shock Proteins (HSP), or chaperonins, of Helicobacter pylori, or a fragment thereof.

18. Proteinaceous material according to claim 17, characterised in that it comprises or consists of HSP A and/or HSP B, having the amino-acid sequence illustrated in Figure 6, or a polypeptide having at least 75 %, and preferably at least 80 % homology with said polypeptide, or a fragment thereof, comprising at least 6 amino-acids.

19. Proteinaceous material according to claim 18 characterised in that it comprises or consists of the HSP A C-terminal sequence :

G S C C H T G N H D H K H A K E H E A C C H D H K K H
or a fragment comprising at least 6 consecutive amino-acids of this sequence.

20. Nucleic acid sequence characterised in that it comprises :

i) a sequence coding for the proteinaceous material of any one of claims 17 to 19 or of any one of the proteinaceous materials of claims 7 to 9 ;
or ii) a sequence complementary to sequence (i) ;
or iii) a sequence capable of hybridizing to sequence (i) or (ii) under stringent conditions ;
or iv) a fragment of any of sequences (i), (ii) or (iii) comprising at least 10 nucleotides.

21. Nucleic acid sequence according to claim 20 characterised in that it comprises all or part of the sequence of plasmid pILL689 (CNCM I-1356), for example the sequence of figure 6, in particular that coding for HSP A and/or HSP B, or a sequence complementary to this sequence, or a sequence capable of hybridizing to this sequence under stringent conditions, or a fragment thereof.

22. Expression vector characterised in that it contains a nucleic acid sequence according to claim 20 or 21.

23. Plasmid pILL689 (CNCM I-1356).

24. Oligonucleotide suitable for use as a primer in a nucleic acid amplification reaction, characterised in that it comprises from 10 to 100 consecutive nucleotides of the sequence of claim 20 or 21.

25. Nucleotide probe, characterised in that it comprises a sequence according to any one of claims 20 or 21 with an appropriate labelling means.

26. Microorganism, stably transformed by an expression vector according to claim 22 or 23.

27. Monoclonal or polyclonal antibodies or fragments thereof, to the proteinaceous material of any one of claims 8 to 10, characterised in that they are either specific for the Helicobacter felis material, or alternatively, cross-react with the gene products of the urease gene cluster of Helicobacter pylori.

28. Monoclonal or polyclonal antibodies according to claim 27 characterised in that they recognise both the Helicobacter felis ure A and/or ure B gene product, and the Helicobacter pylori ure A and/or ure B gene product.

29. Monoclonal or polyclonal antibodies or fragments thereof, to the proteinaceous material of claims 17 or 18, characterised in that they are either specific for the Helicobacter pylori material or, alternatively, cross-react with GroEL-like proteins or GroES-like proteins from bacteria other than Helicobacter.

30. Monoclonal or polyclonal antibodies according to claim 29 characterised in that they recognise specifically the HSP A C-terminal sequence.

31. Use of the immunogenic composition of claim 1 for the preparation of a vaccine suitable for use in man and animals against Helicobacter infection, particularly against Helicobacter pylori and Helicobacter felis.

32. Use of the antibodies of claims 27 to 30 in a therapeutic composition for treating infection by Helicobacter, in particular Helicobacter pylori, Helicobacter heilmannii and Helicobacter felis in man or animals.

33. Method for the production of a pharmaceutical composition according to claim 6, characterised by culturing a transformed micro-organism according to claim 16, and optionally, also a micro-organism according to claim 26, collecting and purifying the Helicobacter urease polypeptide material and where applicable, also the HSP material, and combining these materials with suitable excipients, adjuvants and, optionally, other additives.

34. Use of nucleotide sequences of any claim 15 or 25 for the in vitro detection in a biological sample, of an infection by Helicobacter, optionally following a gene amplification reaction.

35. Kit for the in vitro detection of Helicobacter infection, characterised in that it comprises :

- a nucleotide probe according to claim 15 or 25 ;

- an appropriate medium for carrying out a hybridisation reaction between the nucleic acid of Helicobacter and the probe ;

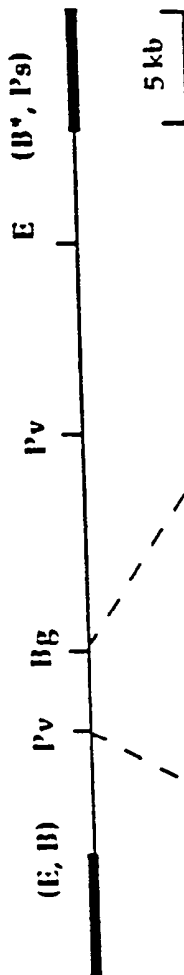
- reagents for the detection of any hybrids formed.

36. Proteinaceous material characterised in that it comprises a fusion or mixed protein including at least one sub-unit of a urease structural polypeptide from Helicobacter pylori or fragment thereof, or from Helicobacter felis or fragment thereof as defined in claims 1 to 3, 5, 7 to 9, and or a heat shock proteins (HSP) from Helicobacter or fragment thereof, as defined in claims 17 to 20.

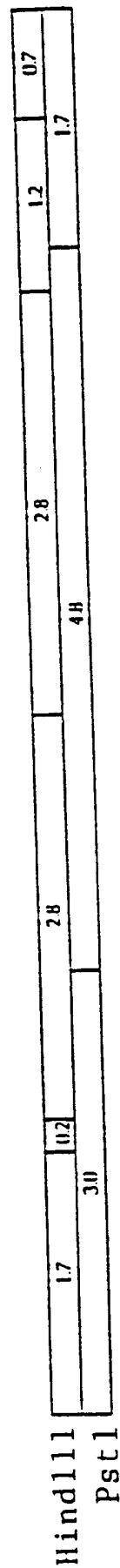
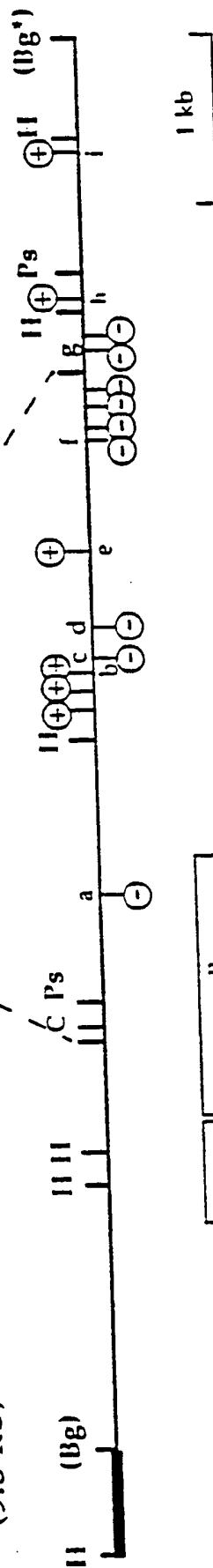
37. Purified antibodies or serum obtained by immunisation of an animal with the immunogenic composition according to claims 1 to 5, or with the proteinaceous material or fragment of claims 7 to 9 or 17 to 19, or with the fusion or mixed protein of claims 36.

38. Kit comprising at least the purified antibodies or serum according to claim 37, and optionally, appropriate media or excipients for administration of the antibodies, or labelling or detection means for the antibodies.

pILL199
(35 kb)



pILL205
(9.5 kb)



- FIGURE 1 -

SUBSTITUTE SHEET (RULE 26)

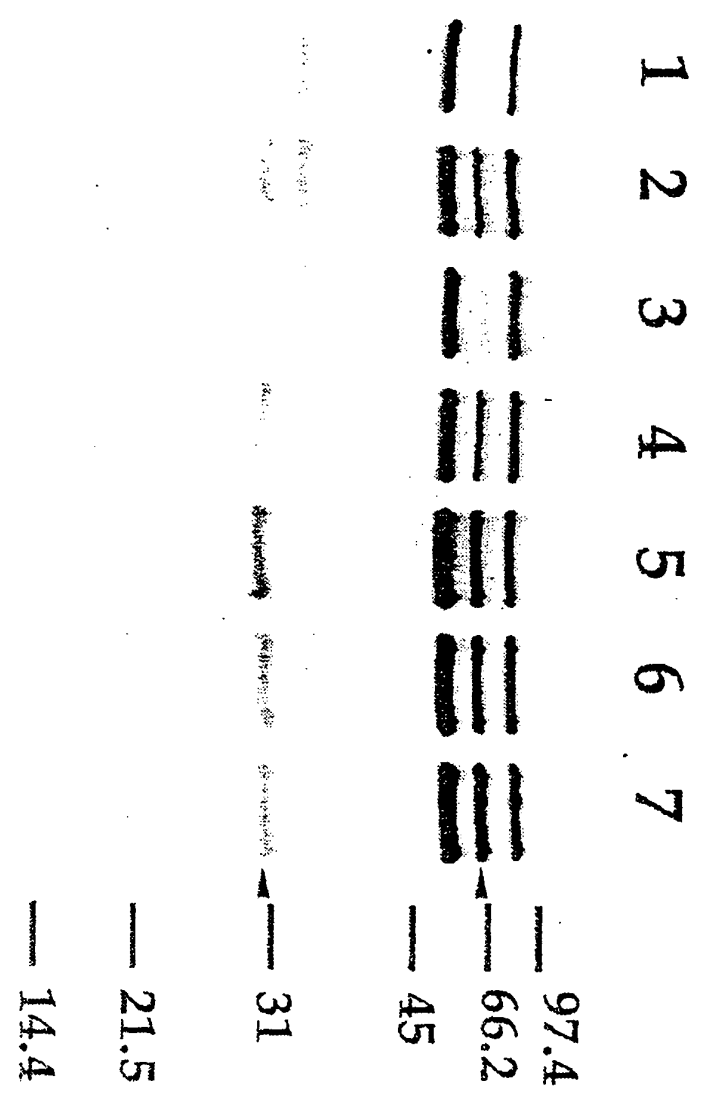


FIGURE 2 A

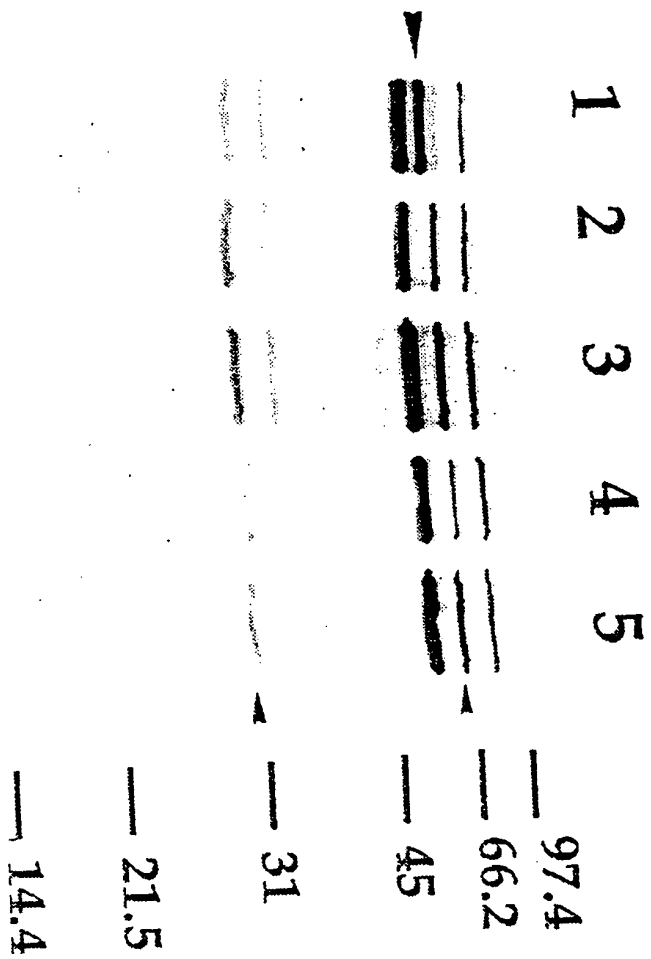


FIGURE 2 B

		31 SD			
1	TGA TAG CTT GGC TAC CAA TAG AAA TTC AAT <u>AAG GAG</u> TTT AGG ATG AAA CTA ACG CCT AAA				
				urea	Met lys leu thr pro lys
61/7	GAA CTA GAC AAG TTA ATG CTC CAT TAT GCG GGC AGA TTG GCA GAA GAA CGC TTG GCG CGT	91/17			
	glu leu asp lys leu met leu his tyr ala gly arg leu ala glu glu arg leu ala arg	151/37			
121/27	GGT GTG AAA CTC AAT TAC ACC GAA GCG GTC GCG CTC ATT AGC GGG CGT GTG ATG GAA AAG				
	gly val lys leu asn tyr thr glu ala val ala leu ile ser gly arg val met glu lys	211/57			
181/47	GCG CGT GAT GGT AAT AAA AGC GTG GCG GAT TTG ATG CAA GAA GGC AGG ACT TGG CTT AAA				
	ala arg asp gly asn lys ser val ala asp leu met gln glu gly arg thr trp leu lys	271/77			
241/67	AAA GAA AAT GTG ATG GAC GGC GTA GCA AGC ATG ATT CAT GAA GTG GGG ATT GAA GCT AAC				
	lys glu asn val met asp gly val ala ser met ile his glu val gly ile glu ala asn	331/97			
301/87	TTC CCC GAT GGA ACC AAG CTT GTA ACT ATC CAC ACT CCG GTA GAG GAT AAT GGC AAA TTA				
	phe pro asp gly thr lys leu val thr ile his thr pro val glu asp asn gly lys leu	391/117			
361/107	GCC CCC GGC GAG GTC TTC TTA AAA AAT GAG GAC ATT ACT ATT AAC GCC GGC AAA GAA GCC				
	ala pro gly glu val phe leu lys asn glu asp ile thr ile asn ala gly lys glu ala				

- FIGURE 3 (1) -

5/56

421/127 451/137
 ATT AGC TTG AAA GTG AAA AAT AAA GGC GAT CGT CCT GTG CAG GTG GGA TCA CAT TTC CAC
 ile ser leu lys val lys asn lys gly asp arg pro val gln val gly ser his phe his
 481/147 511/157
 TTC TTC GAA GTG AAT AAG CTC TTG GAC TTC GAT CGC GCA AAA AGC TTT TGC AAA CGC CTA
 phe phe glu val asn lys leu leu asp phe asp arg ala lys ser phe cys lys arg leu
 541/167 571/177
 GAC ATT GCA TCT GGA ACA GCG GTG CGC TTT GAA CCC GGG GAG GAA AAA AGT GTG GAA CTC
 asp ile ala ser gly thr ala val arg phe glu pro gly glu lys ser val glu leu
 601/187 631/197
 ATT GAC ATC GGC GGC AAT AAG CGC ATC TAT GGC TTT AAT TCT TTG GTG GAT CGC CAA GCC
 ile asp ile gly gly asn lys arg ile tyr gly phe asn ser leu val asp arg gln ala
 661/207 691/217
 GAT GCC GAT GGT AAA AAA CTC GGC TTA AAA CGC GCT AAA GAA GAA GGT TTT GGG TCT GTA
 asp ala asp gly lys lys leu gly leu lys arg ala lys glu lys gly phe gly ser val
 721/227 751 SD
 AAC TGC GGT TGT GAA GCG ACT AAA GAT AAA CAA TAA GGA AAA ACC ATG AAA AAG ATT TCA
 asn cys gly cys glu ala thr lys asp lys gln OCH ureB Met lys lys ile ser
 781/6 811/16
 CGA AAA GAA TAT GTT TCT ATG TAT GGT CCC ACT ACC GGG GAT CGT GTT AGA CTC GGC GAC
 arg lys glu tyr val ser met tyr gly pro thr thr gly asp arg val arg leu gly asp

- FIGURE 3 (ii) -

841/26 871/36
ACT GAT TTG ATC TTA GAA GTG GAG CAT GAT TGC ACC ACT TAT GGT GAA GAG ATC AAA TTT
thr asp leu ile leu glu val glu his asp cys thr thr thr gly glu glu ile lys phe
901/46 931/56
GGG GGC GGT AAA ACT ATC CGT GAT GGG ATG AGT CAA ACC AAT AGC CCT AGC TCT TAT GAA
gly gly gly lys thr ile arg asp gly met ser gln thr asn ser pro ser ser tyr glu
961/66 991/76
TTA GAT TTG GTG CTC ACT AAC GCC CTC ATT GTG GAC TAT ACG GGC ATT TAC AAA GCC GAC
leu asp leu val leu thr asn ala leu ile val asp tyr thr gly ile tyr lys ala asp
1021/86 1051/96
ATT GGG ATT AAA GAC GGC AAG ATT GGC AAG GCA GGC AAT AAG GAC ATG CAA
ile gly ile lys asp gly lys ile ala gly ile gly lys ala gly asn lys asp met gln
1081/106 1111/116
GAT GGC GTA GAT AAT AAT CTT TGC GTA GGT CCT GCT ACA GAG GCT TTG GCA GCT GAG GGC
asp gly val asp asn asn leu cys val gly pro ala thr glu ala leu ala ala glu gly
1141/126 1171/136
TTG ATT GTA ACC GCT GGT GGC ATC GAT ACG CAT ATT CAC TTT ATC TCT CCC CAA CAA ATC
leu ile val thr ala gly gly ile asp thr his ile his phe ile ser pro gln gln ile
1201/146 1231/156
CCT ACT GCT TTT GCC AGC GGC GTT ACA ACC ATG ATT GGA GGA GGC ACA GGA CCT GCG GAT
pro thr ala phe ala ser gly val thr thr met ile gly gly thr gly pro ala asp

- FIGURE 3 (iii) -

7/56

1261/166 1291/176
 GGC ACG AAT GCG ACC ACC ATC ACT CCC GGA CGC GCT AAT CTA AAA AGT ATG TTG CGT GCA
 gly thr asn ala thr thr ile thr pro gly arg ala asn leu lys ser met leu arg ala
 1321/186 1351/196
 GCC GAA GAA TAC GCC ATG AAT CTA GGC TTT TTG GCT AAG GGG AAT GTG TCT TAC GAA CCC
 ala glu glu tyr ala met asn leu gly phe leu ala lys gly asn val ser tyr glu pro
 1381/206 1411/216
 TCT TTA CGC GAT CAG ATT GAA GCA GGG GCG ATT GGT TTT AAA ATC CAC GAA GAC TGG GGA
 ser leu arg asp gln ile glu ala gly ala ile gly phe lys ile his glu asp trp gly
 1441/226 1471/236
 AGC ACA CCT GCA GCT ATT CAC CAC TGC CTC AAT GTC GCC GAT GAA TAC GAT GTG CAA GTG
 ser thr pro ala ala ile his his cys leu asn val ala asp glu tyr asp val gln val
 1501/246 1531/256
 GCT ATC CAC ACC GAT ACC CTT AAC GAG GCG GGC TGT GTA GAA GAC ACC CTA GAG GCG ATT
 ala ile his thr asp thr leu asn glu ala gly cys val glu asp thr leu glu ala ile
 1561/266 1591/276
 GCC GGG CGC ACC ATC CAT ACC TTC CAC ACT GAA GGG GCT GGG GGT GGA CAC GCT CCA GAT
 ala gly arg thr ile his thr phe his thr glu gly ala gly gly gly his ala pro asp
 1621/286 1651/296
 GTT ATC AAA ATG GCA GGG GAA TTT AAC ATT CTA CCC GCC TCT ACT AAC CCG ACC ATT CCT
 val ile lys met ala gly glu phe asn ile leu pro ala ser thr asn pro thr ile pro
 1681/306 1711/316
 TTC ACC AAA AAC ACT GAA GCC GAG CAC ATG GAC ATG TTA ATG GTG TGC CAC CAC TTG GAT
 phe thr lys asn thr glu ala glu his met asp met leu met val cys his his leu asp

1741/326 1771/336
 AAA AGT ATC AAG GAA GAT GTG CAG TTT GCC GAT TCG AGG ATT CGC CCC CAA ACT ATC GCG
 lys ser ile lys glu asp val gln phe ala asp ser arg ile arg pro gln thr ile ala
 1801/346 1831/356
 GCT GAA GAC CAA CTC CAT GAC ATG GGG ATC TTT TCT ATC ACC AGC TCC GAC TCT CAG GCT
 ala glu asp gln leu his asp met gly ile phe ser ile thr ser ser asp ser gln ala
 1861/366 1891/376
 ATG GGA CGC GTA GGC GAG GTG ATC ACA CGC ACT TGG CAG ACA GAC AAA AAC AAA AAA
 met gly arg val gly glu val ile thr arg thr trp gln thr ala asp lys asn lys lys
 1921/386 1951/396
 GAG TTT GGG CGC TTG AAA GAG GAA AAA GGC GAT AAC GAC AAC TTC CGC ATC AAA CGC TAC
 glu phe gly arg leu lys glu glu lys gly asp asn asp asn phe arg ile lys arg tyr
 1981/406 2011/416
 ATC TCT AAA TAC ACC ATC AAC CCC GGG ATC GCG CAT GGG ATT TCT GAC TAT GTG GGC TCT
 ile ser lys tyr thr ile asn pro gly ile ala his gly ile ser asp tyr val gly ser
 2041/426 2071/436
 GTG GAA GTG GGC AAA TAC GCC GAC CTC GTG CTT TGG AGT CCG GCT TTC TTT GGC ATT AAG
 val glu val gly lys tyr ala asp leu val leu trp ser pro ala phe phe gly ile lys
 2101/446 2131/456
 CCC AAT ATG ATT ATT AAG GGC GGA TTT ATT GCG CTC TCT CAA ATG GGC GAT GCC AAT GCG
 pro asn met ile ile lys gly gly phe ile ala leu ser gln met gly asp ala asn ala
 2161/466 2191/476
 TCT ATT CCC ACC CCT CAG CCC GTC TAT TAC CGT GAA ATG TTT GGA CAC CAT GGG AAA AAC
 ser ile pro thr pro gln pro val tyr tyr arg glu met phe gly his his gly lys asn

- FIGURE 3 (v) -

9/56

2221/486 2251/496
 AAA TTC GAC ACC AAT ATC ACT TTC GTG TCC CAA GCG GCT TAC AAG GCA GGG ATC AAA GAA
 lys phe asp thr asn ile thr phe val ser gln ala ala tyr lys ala gly ile lys glu
 2281/506 2311/516
 GAA CTA GGG CTA GAT CGC GCG GCA CCG CCA GTG AAA AAC TGT CGC AAT ATC ACT AAA AAG
 glu leu gly leu asp arg ala ala pro pro val lys asn cys arg asn ile thr lys lys
 2341/526 2371/536
 GAC CTC AAA TTC AAC GAT GTG ACC GCA CAT ATT GAT GTC AAC CCT GAA ACC TAT AAG GTG
 asp leu lys phe asn asp val thr ala his ile asp val asn pro glu thr tyr lys val
 2401/546 2431/556
 AAA GTG GAT GGC AAA GAG GTA ACC TCT AAA GCA GCA GAT GAA TTG AGC CTA GCG CAA CTT
 lys val asp gly lys glu val thr ser lys ala ala asp glu leu ser leu ala gln leu
 2461/566 2491
 TAT AAT TTG TTC TAG GAG GCT AAG GAG GGG GAT AGA GGG GGT TAA TTT AGA GGG GAG TCA
 tyr asn leu phe AMB
 2521 2551
 TTG ATT TAC CTT TGC TAG TTT ATA ATG GAT TTA AGA GAG GTT TTT CGT GTT TTA TAC
 2581 2611
 CGC GTT GAA ACC CTC AAA TCT TTA CCA AAA GGA TGG TAA

- FIGURE 3 (vi) -

urea

• • • • •
MKLTPKELDKMLMHHYAGRLAEERLARGVKLN*Y*TEAVALISGRVME
*****E**KRRKEK*J*****V*****AHJ**
E**R*K**L*FT**LV**RR**K*L**P*R**C*AI**
S*R*VE**G**N**Y**QKR**R***T*****ASQI**
=====

H. f.
H. p.
P. m.
J. b.

KARDGNKSVADLMQEGRTWLKKENVMDGVASMIHEVGIENFPDG 89
E**A*K*TA*****L**PDD*****M**** 89
G**E*-T**Q*S*****V*TA**Q**E**PE**KD**QV*CT*** 89
Y*****E**T**Q**CL*QHL*GRRQ*LPA*PHLLNA*QV**TE*** 90

- FIGURE 4 (i) -

H.f. TKLVTHTPV-----•••••
 H.p. *****I-----EDNGKLAPGEV
 P.m. *****S*IV 100-----A*****V***L
 J.b. *****V*D*ISRENGELQEALFGSLLPVPSLDKFAETKEDNRI***I
 =====

•••••
 FLKNEDITI--NAGKEAISLKVKNKGD RP VQVGSHEFFEVNKL 154
 *****--E*K*V*V*****I*****RC* 154
 RVNNAALGD*EL**R*TKTIQ*A*H*****C***Y***EA* 51
 LCED*CL*L--*I*RK*VI**TS*****I*****PY* 180
 =====

- FIGURE 4 (11) -

12/56

H. f. DFDRAKSFCKRLDIASGTAVRFEPEGEEKSV-ELIDIGCNKRIY
H. p. ***E*T*G*****~*****R**F
P. m. R*A*KETLGF*N*PA*M*****QSRT*D**VAFA*KRE**
J. b. T**R*AYGM**N**AG*****DC***-T*VS*E**KV*R

• • • • •	• • • • •	237		
GFNSLVDRQAD	QDGKKLGLKRAKEKF	-GSVNCGCEAT---	KDKQ	
A	***NES***	IA**R**	**AKSDDNYVKTI-*	E
-----	-----	-----	**KVMGKLESE--	K*
*G*A	IA*GPVNETNLEA	MIAVRSR**	-*HEEKDAPEGFT*	EDPNC
				SF-270

- FIGURE 4 (iii) -

ureB

H.f.
H.p.
P.m.
J.b.

```

      . . . . .
MKKISIRKEYVSMYGP TTGDRVRLGDTDLI LEVEHDC
*****K*****A*****Y
**T**A**Q**A**D**P*****L**A**A**R**F**A**I**K**P
NTF**H*****ANK*****K*****I*****A**L**A**I**K**Y
= = = = =

```

```

      . . . . .
TTYGFEIKFGGKTIRDGMSQTNSSPYEL-DLVLTNALIVDYTG I 81
AIAAAAL,*****LE*****S**N**K**E**--**I*****I***** 81
*****V*****V*****G**S**Q**V**A**E**C**V**--**V**L**I*****I**L*****W** 81
AL**A**D**A**C**V*****V*****V*****G**A**S**C**G**H**P**P**A**I**S**I**T**A**I*****V**I***** 352
= = = = =

```

- FIGURE 4 (iv) -

14/56

H. f.

H. p.

P. m.

J. b.

```

.      .      .      .      .      .      .      .      .      .
YKADIGIKDKIAGIGKAGNKDMQDGVDDNNLCVGPATEALAAEGL
*****G*****K***S*****G***
V*****R*****P*VQPN**IVI--**G**VV*G**K
I*****L**S*****P*IMN**FSNMII*AN**VI*G**
===== = === = = = =

```

```

.      .      .      .      .      .      .      .      .      .
IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGCTGPADGNTATT 171
*****A*****A*****A*****A*****A*****A***** 171
*****C***AQEGLV*****FI*****VA***** 169
*****C***Y*C**LVYEAI S**I**LV*****A**R** 442
===== = = = = = = = = =

```

- FIGURE 4 (v) -

H.f.
H.p. ITPGRANLKSMLRAAEYAMNLGFLAKGNVSEPSLRDQIEAGAI
P.m. *****S*****A*NDA*A*****
J.b. V***IW*MYR**E*VD*LPI*V*LFG**CV*QPEAI*E**T***
C**SPTQMRL**OSTDDLPL*F**TG**SS*KPDE*HEI*K**M
==
==

.
GFKIHEDWGSTPAIHHCLNVADEYDVQVAIHTDTLNEAGCVEDT 261
*****T**S**N*A*D**K***** 261
*L*****A**M**N*****M*****S*****G*FY*E* 259
*L*L*****DN**TI*EH**I*IN*****F**HS 532
==
==
==
==
==
==

- FIGURE 4 (vi) -

H. f. I.EAIGRTIITFTTEGAGGGHAPDVIKMAGEFNILPASTNPTIFE
H. p. MA***TM***I**V**I***
P. m. VK***VI***SV**P***M*Y
J. b. IA*FK**TI**Y*S***I**VC*IK*V*S***R*L.

TKNTEAEHMDMLMVCIIHLDKSIKEDVQFADSRIRPQTIAAEDQLH	351
V*****T**	351
I**VD**I,*****P**P**A**F*****RE*****I**	349
S**ID**I,*****RE**P**A**H*****KK*****V**	622

- (iii) -
- (iv) -

```

H.f.      . . . . .
          DMGIFSITSSDSQAMGRVGEVITRTWQTADKNKKEFGRLKEEKGD
H.p.      *****
          *****
P. m.      ***AI*VM*****L***C*H***LQR*T*AGDSA*
          *I*AI**I*****S*****AQT*P**CDSS*
          = = = = =

```

```

NDNFRIKRYISKYTINGIAHGISDYVGSVEVGKYADLVLSWPAF 441
*****L*****A*****E*****V***** 441
*****N*****A*****AL*****AHT*****I*K**L**I***D*** 439
*****A*****A*****Q*****L*****K*S* 712
=====

```

- FIGURE 4 (viii) -

H. f. FGIKPNMI IKGGFIALSQMGDANASIP TPQPVYYREMFIIHGKNK
H. p. **V*****
P. m. **V**AL**MVRYP**I**A*****P**YACL**A**
J. b. **T**E**V**MVAWADI**P*****KM*P*Y*TL**AG

.
 FDTNITFVSQAAAYKAGIKEELGLDRAAPPVKN--CRNITKKDLKF 529
 Y*R*****DK*****E*QVL*****MQ* 529
 YQ*SMI*M*K*GIEA*VP*K**KSLSLGRVEGC*H***ASMIH 529
 GALS*A**K**LDQRVNVLY**NKRVEA*S*--V*KL*L*M*L 800

- FIGURE 4 (ix) -

19/56

H.f.	NDVT	AI	ID	VN	PE	TY	KV	KG	KE	VT	SK	AA	DE	LS	LA	Q	L	Y	N	I	F	569
H.p.	**T	**E	**H	**F	**H	**F	**H	**F	**H	**F	**H	**F	**H	**F	**H	**F	**H	**F	**H	**F	**H	569
P. m.	*NY	VP	*EL	D	*Q	**I	**A	**V	PL	VC	EP	*T	**P	M	**R	*F	**					569
J.b.	**AL	PE	*T	*D	**S	*T	**A	**L	LC	VS	E	*T	T	V	P	*S	R	N	*F	**		840

uraA :74	%	Identity	uraB :88	%	Identity
uraA :46	%	Identity	uraB :62	%	Identity
uraA :47	%	Identity	uraB :59	%	Identity

FIGURE 4 (x) -

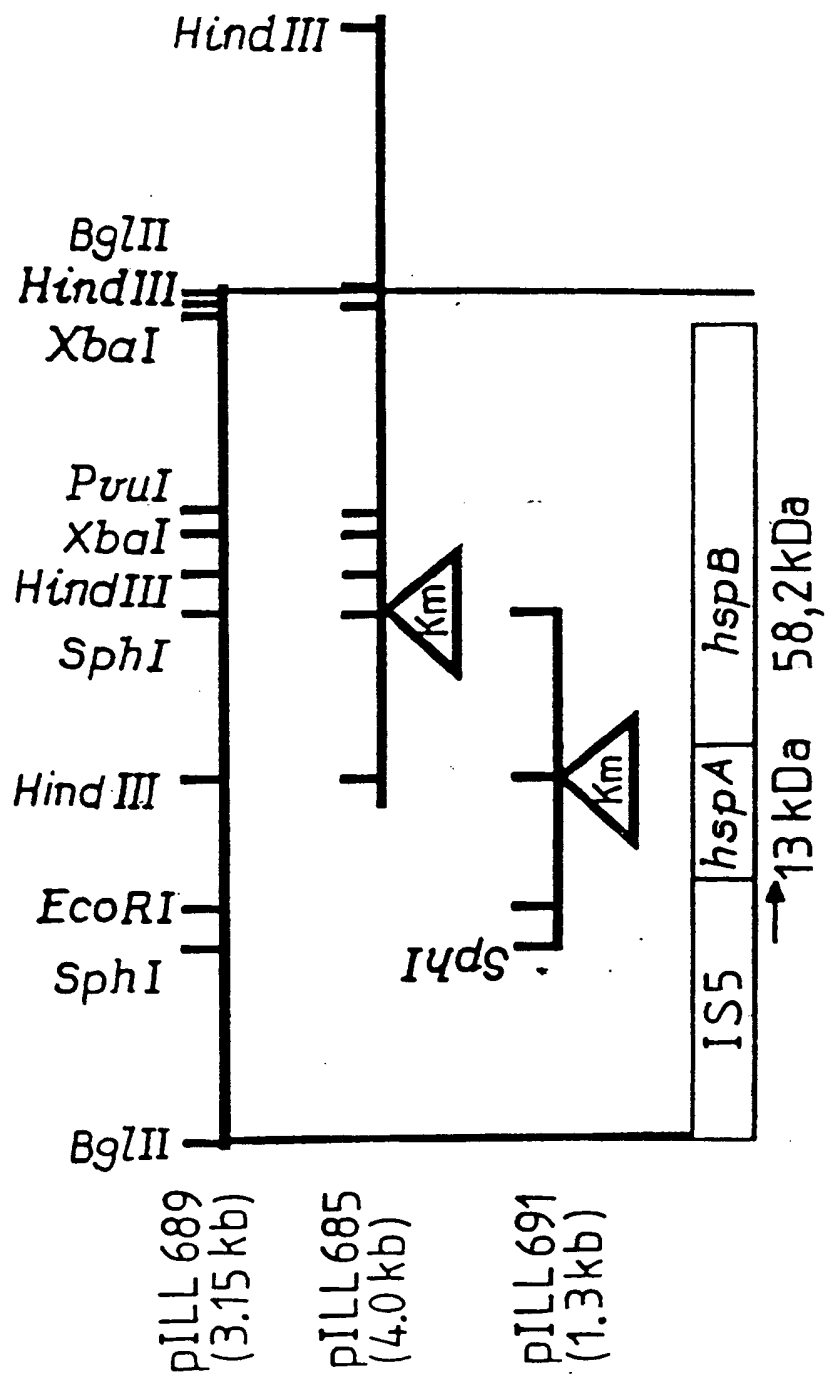


FIGURE 5

21/56

1 ACA AAC ATG ATC TCA TAT CAG GGA CTT GTT CGC ACC TTC CCT AAA AAT GCG CTA TAG TTG
 31
 61 TGT CGC TTA AGA ATA CTA AGC GCT AAA TTT CTA TTT TAT TTA TCA AAA CTT AGG AGA ACT
 91
 121 GAA ATG AAG TTT CAA CCA TTA GGA GAA AGG GTC TTA GTA GAA AGA CTT GAA GAA GAG AAC
 151/10
 met lys phe gln pro leu gly glu arg val leu val glu arg leu glu glu asn
 181/21
 AAA ACC AGT TCA GGC ATC ATC ATC CCT GAT AAC GCT AAA GAA AAG CCT TTA ATG GGC GTA
 211/31
 lys thr ser ser gly ile ile ile pro asp asn ala lys glu lys pro leu met gly val
 241/41
 GTC AAA GCG GTT AGC CAT AAA ATC AGT GAG GGT TGC AAA TGC GTT AAA GAA GGC GAT GTG
 271/51
 val lys ala val ser his lys ile ser glu glu gly cys lys cys val lys glu gly asp val
 301/51
 ATC GCT TTT GGC AAA TAC AAA GGC GCA GAA ATC GTT TTA GAT GGC GTT GAA TAC ATG GTG
 331/71
 ile ala phe gly lys tyr lys gly ala glu ile val leu asp gly val glu tyr met val
 361/71
 CTA GAA CTA GAA GAC ATT CTA GGT ATT GTG GGC TCA GGC TCT TGC TGT CAT ACA GGT AAT
 391/91
 leu glu leu glu asp ile leu gly ile val gly ser gly ser cys cys his thr gly asn
 421/91
 CAT GAT CAT AAA CAT GCT AAA GAG CAT GAA GCT TGC TGT CAT GAT CAC AAA AAA CAC TAA
 451/111
 his asp his lys his ala lys glu his glu ala cys cys his asp his lys lys his OCH
 481
 AAA ACA TTA TTA TTA AGG ATA CAA AAT GGC AAA AGA
 511

- FIGURE 6 (1) -

22/56

479 AAA CAT TAT TAT TAA GGA TAC AAA ATG GCA AAA GAA ATC AAA TTT TCA GAT AGC GCA
 509/2 met ala lys glu ile lys phe ser asp ser ala
 539/12 AGA AAC CTT TTA TTT GAA GGC GTA AGA CAA CTC CAT GAC GCT GTC AAA GTA ACC ATG GGG
 arg asn leu leu phe leu glu gly val arg gln leu his asp ala val lys val thr met gly
 569/22
 599/32 CCA AGA GGC AGG AAC GTG TTG ATC CAA AAA AGC TAT GGC GCT CCA AGC ATC ACC AAA GAC
 pro arg gly arg asn val leu ile gln lys ser tyr gly ala pro ser ile thr lys asp
 629/42
 659/52 GGC GTG AGC GTG GCT AAA GAG ATT GAA TTA AGT TGC CCC GTG GCT AAC ATG GGC GCT CAG
 gly val ser val ala lys glu ile glu leu ser cys pro val ala asn met gly ala gln
 689/62
 719/72 CTC GTT AAA GAA GAT GCG AGC AAA ACC GCT GAT GCC GCC GGC GAT GGC ACG ACC ACA GCG
 leu val lys glu asp ala ser lys thr ala asp ala ala ala gly asp gly thr thr ala
 749/82
 779/92 ACC GTG CTG GCT TAT AGC ATT TTT AAA GAG GGC TTG AGG AAT ATC ACG GCT GGG GCT AAC
 thr val leu ala tyr ser ile phe lys glu gly leu arg asn ile thr ala gly ala asn
 809/102
 839/112 CCT ATT GAA GTG AAA CGA GGC ATG GAT AAA GCG CCT GAA GCG ATC ATT AAT GAG CTT AAA
 pro ile glu val lys arg gly met asp lys ala pro glu ala ile ile asn glu leu lys
 869/122
 899/132 AAA GCG AGC AAA AAA GTG GGC GGT AAA GAA GAA ATC ACC CAA GTA GCG ACC ATT TCT GCA
 lys ala ser lys lys val gly gly lys glu glu ile thr gln val ala thr ile ser ala
 929/142

- FIGURE 6 (11) -

- FIGURE 6 (iii) -

24/56

1379/292 1409/302
 GCT GTT TTA ACC GGC GGT CAA GTC ATT AGC GAA GAA TTG GGC TTG AGT CTA GAA AAC GCT
 ala val leu thr acc gly gly gln val ile ser glu glu leu gly leu ser leu glu asn ala

1439/312 1469/322
 GAA GTG GAG TTT TTA GGC AAA GCG AAG ATT GTG ATT GAC AAA GAC AAC ACC ACG ATC GTA
 glu val glu phe leu gly lys ala lys ile val ile asp lys asp asn thr thr ile val

1499/332 1529/342
 GAT GGC AAA GGC CAT AGC CAT GAC GTC AAA GAC AGA GTC GCG CAA ATC AAA ACC CAA ATT
 asp gly lys gly his ser his asp val lys asp arg val ala gln ile lys thr gln ile

1559/352 1589/362
 GCA AGC ACG ACA AGC GAT TAC GAC AAA GAA GAA TTG CAA GAA AGA TTG GCC AAA CTC TCT
 ala ser thr thr ser asp tyr asp gly ala ala ser glu gln glu arg leu ala lys leu ser

1619/372 1649/382
 GGC GGT GTG GCT GTG ATT AAA GTG GCG GCT GCG AGT GAA GTG GAA ATG AAA GAG AAA AAA
 gly gly val ala val ile ile lys val gly ala ala ser glu val glu met lys glu lys lys

1679/392 1709/402
 GAC CGG GTG GAT GAC GCG TTT AGC GCG ACT AAA GCG GCG GTT GAA GAA GGC ATT GTG ATT
 asp arg val asp asp ala leu ser ala thr lys ala ala val glu glu gly ile val ile

1739/412 1769/422
 GGG GGC GGT GCG GCC CTC ATT CGC GCG GCC CAA AAA GTG CAT TTG AAT TTA CAC GAT GAT
 gly gly gly ala ala ala ile arg ala ala gln lys val his leu asn leu leu his asp asp

- FIGURE 6 (iv) -

26/56

MAKEIKFSDSARNLLEFEGVRQLHDAVKVTMGPRGRNVLIQKSYG
 LR*G*D**LQMLA**NA*A**Q**VLE***
 MA**DV**GND**VKMLR**NV*A*****L**K*****VLD**F*
 MA**N**YNED**KKIHK**KT*AE*****L**K**H*V*D**F*
 T*AYDEERG*ER*LNS*A*****L**K*****VLE*KW*
 Y**DV-**GAD**ALMLQ**DL*A**A*****K**T*I*EQ*W*

APSITKDGVSVAKEIELSCPVANMGAQLVKE:DASKTADAAGDG
 TV***FEHREM*****M***V*****S*T*****
 T***R*****EDKFE*****M***V***AN*****
 S*QV*****T*****EDKHE*****M***V*****K*****
 TN***I*****ED*YEKI**F*****V*K**D*V*****
 S*KV*****T**S*D*KDKYK*I**K**QDV*NN*NEE*****

- FIGURE 7 A (i) -

27/56

TTTATVLAYSIFKEGLRNITAGANPIEVKRGMDKAPEAIINELKK
 *****R**LV**HKAVA**M**MDL**I**VL*VTKK*QA
 *****QA*IT**KAVA**M**MDL**I**VT*AVE**A
 *****EA*YS**V**MLD**I**VKVVVD*I**
 *****QALV**VA**LGL**IE**VDKVTET*L*
 *****R**A**FEK*SK**VEIR**V*L*VD*V*A**

ASKKVGGKEITQVATISANSNDHNI GKL IADAMEKVKG DGVIT
 M**PCKDSKA**A**G**EA**AI**E**E**E**E**E**E**
 L*VPCSDSKA**A**G**ETV**E**E**D**E**E**E**E**
 I**P*QHIIK**A**N*AE**N**E**N**S**
 DA*E*ET**Q*AAT*A**G*QS**D**E**D**NE**
 Q**P*TTP**A**G*KE**NI*SD**K**RK**

28/56

VEEAKGIEDELDVVEGMQFDRGYLSPYFVTNAEKMTAQLDNAYIL
 DGN*L*NY*****I**IN*QQN*SCE*EHPE**
 DGT*LQ***INKP*TGAVE*ESPF**
 ****F*TV*****N*****S**P*TQECV*EE*LV**
 SNTFGLQ*ELTR**K**I*G***D**RQE*V*EEP***
 *KDG*TLN**EII**K*****I**INTSKGQKCEFQD**V*

LTDK¹KISSMKDILPLLEKTMKEGKPLLI²AE³DIEGEALTTLVV
 *V**V**IREM*SV**GVA*S*R*****I*****A***
 *A***NIREM**V**AVA*A*****V*****A**
 IY*****GI**F**V*QQVAES*R*****A***
 *VSS*V*TV**L*****VIQA**S*****V*****S***
 *SE*****IQS*V*A**IANLV⁴LN⁵R*KVGLQVAVK*PGF**L

- FIGURE 7 A (III) -

29/56

NKLRGVI, NIAAVKAPGFGDRRKEMI, KDIAVLTGGQVISEELGLSL
 *NM**IVKVC*****A**Q**I**K*****I**K**
 *TI**IVKV*****A**Q**T*****T*****I**ME*
 *R**AGFRVC*****A**E**I*****L*****MK*
 *I**TFKSV*****A**Q**M**I***A*****V**T*
 *R*KVG*QVV*V*****N**NQ**K**M**IA***A*FG**GLTLN

ENAEVEF-LGKAKI-VIDKDNTTIVDGKGSHDVKDRVAQIKT
 *G*TL*D-*S**RI*VT**E***I**E**KATEINA*I**RA
 *K*TL*D-*Q**RV**N**T***I**V**EEAAIQG*****RQ
 TTLAM-***KVIVS*ED*****E*L*SKE*IES*CES**K
 TDLSL-***RKV*MT**E*****E*A*DTDAIAG*****R*
 LEDVQPHD***VGEVIVT**DAMLLK*K*DKAQIEK*IQE*IE

- FIGURE 7 A (iv) -

30/56

QIASTSDYDKEKLQERLAKLSGGVAVIKVGAASEVEMKEKCD
 *MEE*****R*****V*****A*****T*****A
 EEA***R*****V*****A*****T*****A
 EDS***R*****V*****A*****T*****I*****
 E*ENSD*****R*****A*****A*****T*****L*****R*H
 *LDV***E*EK**N*****SD*****L***GT*D**VN*****

RVDDALSATKAAVEEGIVIGGGAALIRAAQKVH--LN-LHDDEK
 E*H**R*****A***V*****QKALDS--*KGDN**QN
 E*H**R*****V*A***V*****V*S*LAD--*RGQNE*QN
 QHA*L**LP*****T**V*CIP'TLEAFIPILTNE**Q
 *IE**VRNA*****A***VT*LQ**PALDK--*K-*TG**A
 T*N**R*****L***C**L*CIPALDS--*TPANE*Q*

- FIGURE 7 A (v) -

31/56

VGYEII MRAIKAPLAQIAINAGYDGGVVNEVEKHEGHEGFNA
 M*IN*LR**ES*MR**VT***EAS***K*AE*KDNY***
 IKVALME***R**VL*C*EEPS**A*T*KGGD*NY**Y**
 I*AR*VLK*LS***K***A***KE*AIICQQ*LSRSSSE*YD*
 T*AN*VKV*LE***K***F*S*MEP***AEK*RNLSVGH*L**
 I*I***K*TL*I*AMT***K***V***SLI*EKIMQSSSEVGYD*

SNGKYVDMFKEGIDPLKVERIALQNAVSVSSLLLTTEATVHEIK
 AT*E*G**VEM**L**T**T**M***A**A**M***CM*ADLP
 ATEE*GN*IDM**L**T**T**S***Y*A**AG*MI***CM*TDLP
 LRDA*T**IEA**L**T**T**C**ES*A**AG***LIAD*P
 AT*EYE*LL*A*VA**V**T**S***A*IAG*F***V*ADKP
 MA*DF*N*VEK***T**V**T**LD*A**A***T*A*VV**T**P

32/56

EEKAAPAMPDMGGMGGMGGMGMM	HspB <i>Helicobacter pylori</i>
KKEEGVGAG*****	HtpB <i>Legionella pneumophila</i>
KND**-DLGAA*****	GroEL <i>Escherichia coli</i>
***SSSA-*A*P*A*-*DY	HypB <i>Chlamydia psittaci</i>
*KT*****SDPTGGMGGMDF	GroEL1 <i>Mycobacterium leprae</i>
D-*G*GA**-M**G*F	63 kDa Human mitochondrial protein P1

Identity : 62.7%
 60.5%
 59.6%
 57.4%
 42.5%

Comparison of the GroEL-like proteins from various bacteria

33/56

35%	<i>Helicobacter pylori</i>	MKFQPLGERVL
	<i>Mycobacterium leprae</i>	**EDKI*
35.6%	<i>Legionella pneumophila</i>	**IR**HD**V
33.8%	<i>Thermophilic bacterium</i>	*LK-***D*IV
32.2%	<i>Clostridium perfringens</i>	*SIK***D**V
20.3%	<i>Escherichia coli</i>	MNIR**HD**I

VERLEENKTSSGIIIPDNAKEKPLMGVVKAV--SHKI
*QAG*A*TM*P**LV**ED*****QE*T*V**GPGRWDE
*R*M**RT*AG**V**S*T***MR*EI**GAGKVLE
I*VV*T***A***VL**T***QE*R*V**GAGRVL
IK**A*ET*K*VTGT***R*QEAE*V**GPGAIVD
*K*K*V*T*SAG**VLTGS*AA*STR*E*L**GNRILE

- FIGURE 7 B (1) -

34/56

SEGCKC---VKEGDVIAFGKYKGAEIVLDGVEYMVLELE
 DGAKRIPVD*S**IVIYS**G*T**KYN*E**LI*SAR
 NGDVRA---**V***VL***S*T**V**K*LV**MRED
 NGQRIGRKS-*V**RVI*S**A*T**VKY**K*Y*I*RES
 -GKRTEME-*I**KVLYS**A*T**VKFE*E**TI*RQD
 NGEVKP-LD**VG*IVI*NDGY*VKSEKIDN*EVLIMS*

DILGIVGSGSCCHTGNHDKHAKHEACCHDHKKH
 *V*AV*SK
 MVIEK
 **AVIR
 ***A**E
 SDILAIVEA

Comparison of the GroES-like proteins from various bacteria

- FIGURE 7 B (ii) -

35/56

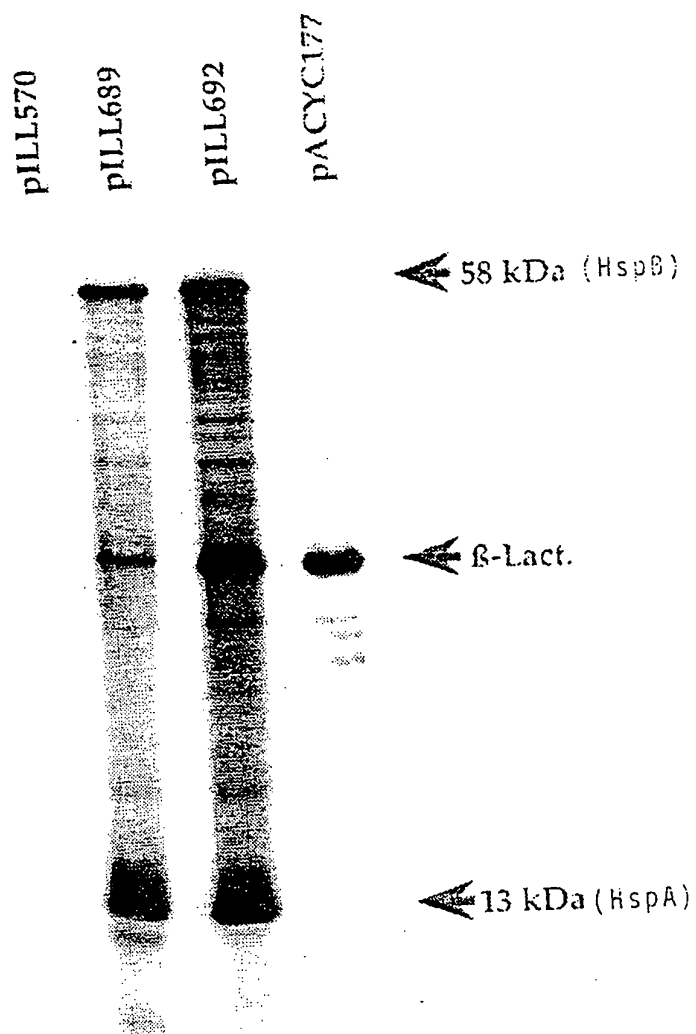


FIGURE 8

36/56

1/1
ATG TTA GGT CTT GTG TTA TTG TAT GTT GCG
Met leu gly leu val leu leu tyr val ala

31/11
GTC GTG CTG' ATC AGC AAC GGA GTT AGT GGG
val val leu ile ser asn gly val ser gly

- FIGURE 9 (i) -

37/56

61/21
CTT GCA AAT GTG GAT GCC AAA AGC AAA GCC
leu ala asn val asp ala lys ser lys ala

91/31
ATC ATG AAC TAC TTT GTG GGG GGC GAC TCT
ile met asn tyr phe val gly gly asp ser

- FIGURE 9 (II) -

121/41
CCA TTG TGT GTA ATG TGG TCG CTA TCA TCT
pro leu cys val met trp ser leu ser ser

151/51
TAT TCC ACT TTC CAC CCC ACC CCC CCT GCA
tyr ser thr phe his pro thr pro pro ala

181/61
ACT GGT CCA GAA GAT GTC GCG CAG GTG TCT
thr gly pro glu asp val ala gln val ser

211/71
CAA CAC CTC ATT AAC TTC TAT GGT CCA GCG
gln his leu ile asn phe tyr gly pro ala

241/81
ACT GGT CTA TTG TTT GGT TTT ACC TAC TTG
thr gly leu leu phe gly phe thr tyr leu

271/91
TAT GCT GCC ATC AAC AAC ACT TTC AAT CTC
tyr ala ala ile asn asn thr phe asn leu

41/56

301/101
GAT TGG AAA CCC TAT GGC TGG TAT TGC TTG
asp trp lys pro tyr gly trp tyr cys leu

331/111
TTT GTA ACC ATC AAC ACT ATC CCA GCG GCC
phe val thr ile asn thr ile pro ala ala

- FIGURE 9 (vi) -

42/56

361/121
ATT CTT TCT CAC TAT TCC GAT GCG CTT GAT
ile leu ser his tyr ser asp ala leu asp

391/131
GAT CAC CGC CTC TTA GGA ATC ACT GAG GGC
asp his arg leu leu gly ile thr glu gly

- FIGURE 9 (vii) -

421/141
GAT TGG TGG GCT TTC ATT TGG CTT GCT TGG
asp trp trp ala phe ile trp leu ala trp

451/151
GGT GTT TTG TGG CTC ACT GGT TGG ATT GAA
gly val leu trp leu thr gly trp ile glu

44/56

481/161
TGC GCA CTT GGT AAG AGT CTA GGT AAA TTT
cys ala leu gly lys ser leu gly lys phe

511/171
GTT CCA TGG CTT GCC ATC GTC GAG GGC GTG
val pro trp leu ala ile val glu gly val

- FIGURE 9 (ix) -

541/181
ATC ACC GCT TGG ATT CCT GCT TGG CTA CTC
ile thr ala trp ile pro ala trp leu leu

571/191
TTT ATC CAA CAC TGG TCT TGA
phe ile gln his trp ser OPA

Comparison of the amino acid sequence of the Urel proteins deduced from the nucleotide sequence of the *urel* gene of *H. felis* and that of *H. pylori*

Percent Similarity : 88.2
Percent Identity : 73.8

First line : *H. felis* Urel
Second line : *H. pylori* Urel

```

1  KGWMI.GI.VI.I.YVAVV.I.TSNGVSGLANVDAKSKAIMNYFVGDSPLCVMWS  50
   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1  . . . M I G L V L L Y V G I V L I S N G I C G L T K V D P K S T A V M N F V G G L S I I C N V . V  46

51  LSSYSTFHPPTPPATGPEDVAQVSQHLINIFYGPATGLLFGFTYLYAAINNIT  100
   : . | | . : | | : | | : | | : | | : | | : | | : | | : | | : | |
47  VITYSALNPTAPVEGAEDIAQVSHHLLTNFYGPATGLLFGFTYLYAAINNIT  96

101  ENLDWKPYGWYCLFVTINTIPAAILSHYSDALDDHRLLLGITEGDWWAFIW  150
   | . | | | : | | : | | . | | | | | | | | | | | | | | | | | |
97  FGLDWRPYSWYSLEVAINTIPAAILSHYSDMLDDHKVLGI'TEGDWWAIIW  146

151  LAWGVLWL'GWIECALGKSIGKFVPWLAI'VEGVI'AWIPAWLL'FIQIHS  199
   | | | | | | | | : | | | . | | | | | | | | | | | | | | | | |
147  LAWGVLWL'TAFIENTILKIPLGKF'IPWLAI'IEGIL'AWIPAWLL'FIQIHW  195

```

The Genetic Code

		Second Position				
		U	C	A	G	
First Position (5' End)	U	UUU } Phe	UCU } Ser	UAU } Tyr	UGU } Cys	U
		UUC } Phe	UCC } Ser	UAC } Tyr	UGC } Cys	C
		UUA } Leu	UCA } Ser	UAA* Stop	UGA* Stop	A
		UUG } Leu	UCG } Ser	UAG* Stop	UGG Trp	G
	C	CUU } Leu	CCU } Pro	CAU } His	CGU } Arg	U
		CUC } Leu	CCC } Pro	CAC } His	CGC } Arg	C
		CUA } Leu	CCA } Pro	CAA } Gln	CGA } Arg	A
		CUG } Leu	CCG } Pro	CAG } Gln	CGG } Arg	G
	A	AUU } Ile	ACU } Thr	AAU } Asn	AGU } Ser	U
		AUC } Ile	ACC } Thr	AAC } Asn	AGC } Ser	C
		AUA } Ile	ACA } Thr	AAA } Lys	AGA } Arg	A
		AUG* Met	ACG } Thr	AAG } Lys	AGG } Arg	G
	G	GUU } Val	GCU } Ala	GAU } Asp	GGU } Gly	U
		GUC } Val	GCC } Ala	GAC } Asp	GGC } Gly	C
		GUA } Val	GCA } Ala	GAA } Glu	GGA } Gly	A
		GUG* Val	GCG } Ala	GAG } Glu	GGG } Gly	G

Third Position (3' End)
- FIGURE 11 -

Abbreviations for amino acids

<i>Amino acid</i>	<i>Three-letter abbreviation</i>	<i>One-letter symbol</i>
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Asparagine or aspartic acid	Asx	B
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glutamine or glutamic acid	Glx	Z
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

49/56

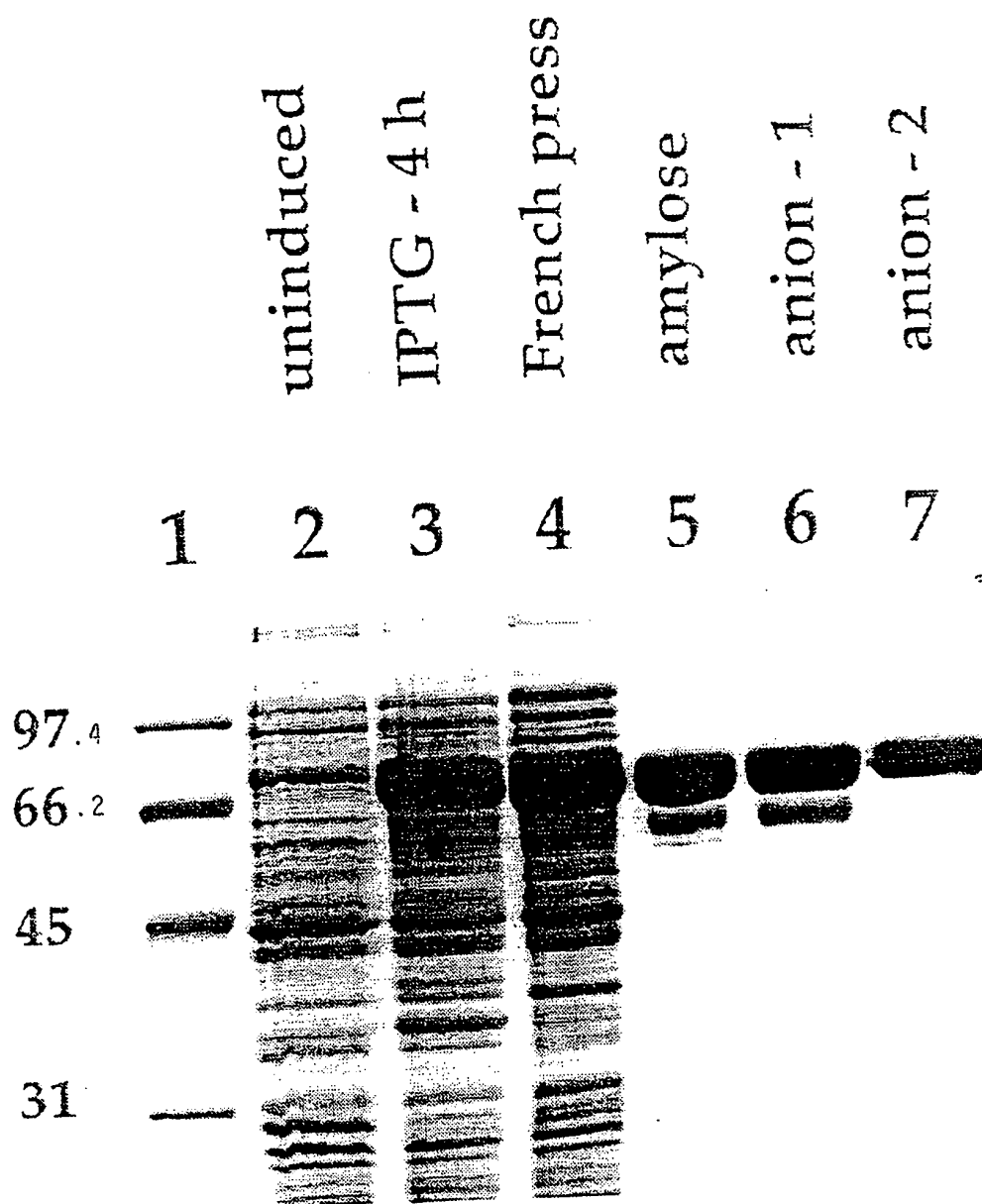
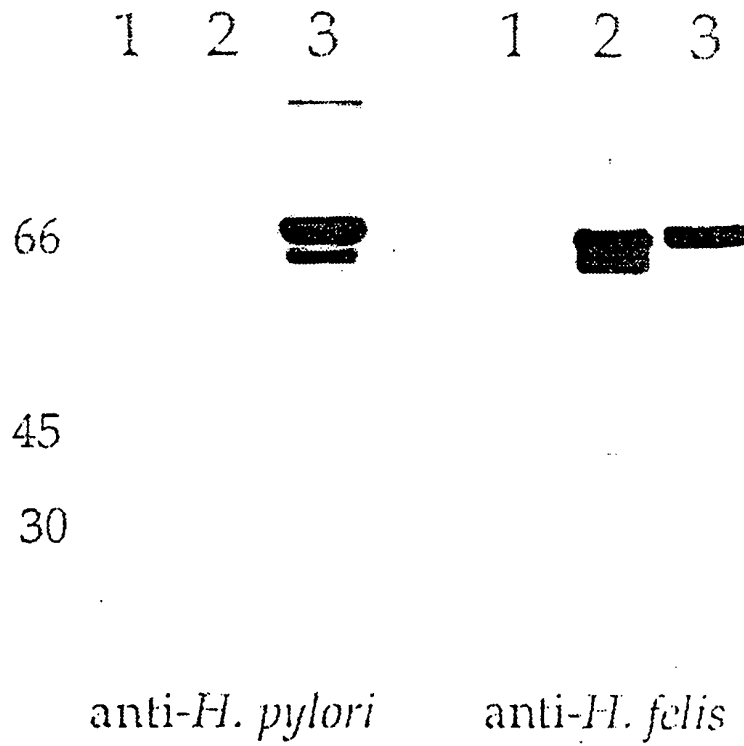


FIGURE 13

50/56

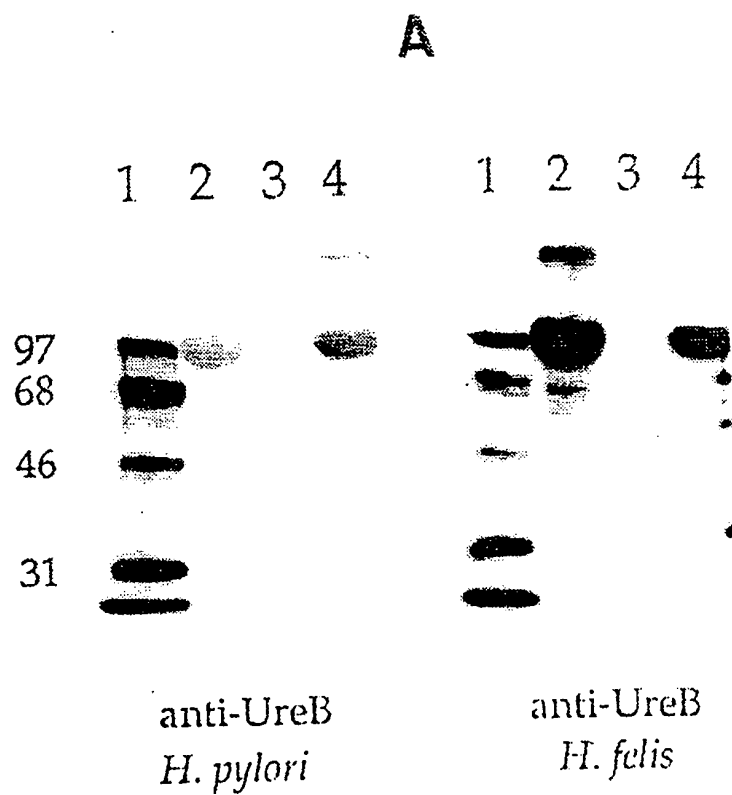
FIGURE 14



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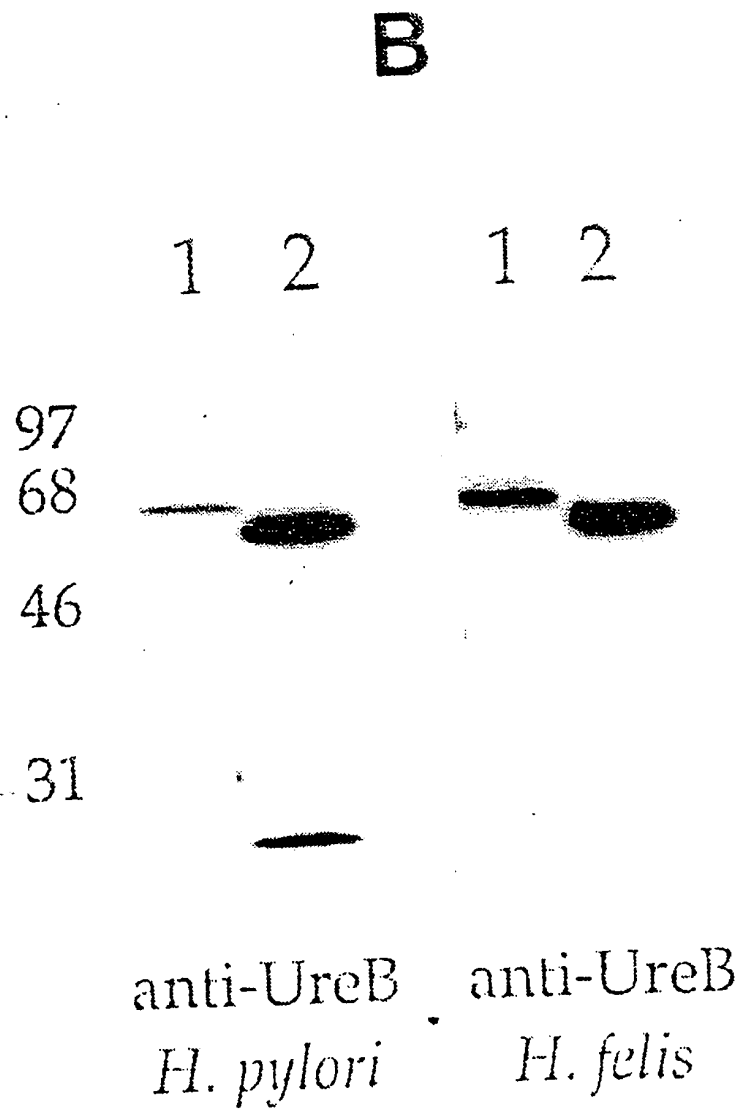
51/56

FIGURE 15



54/56

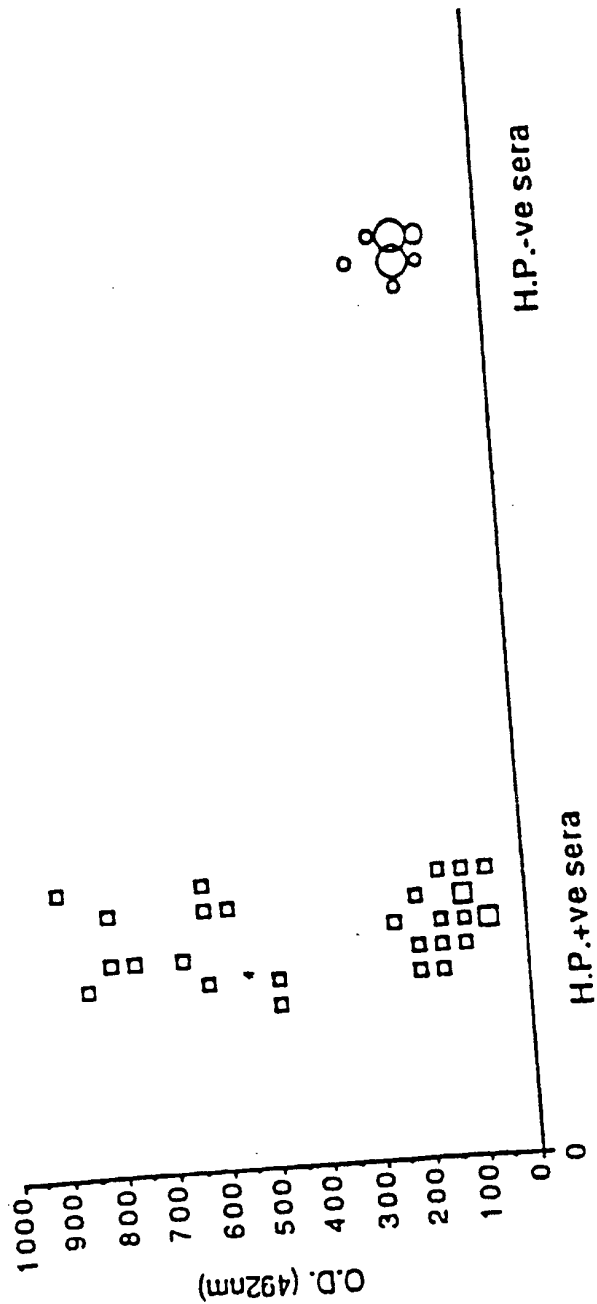
FIGURE 16



53/56

FIGURE 17(i)

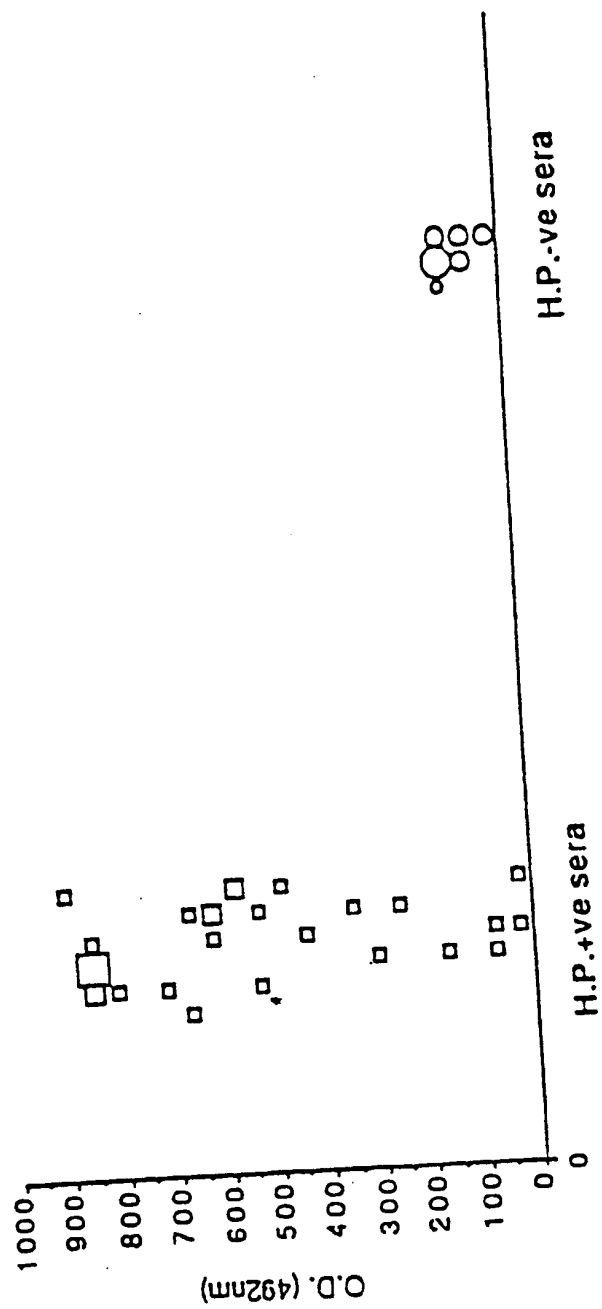
HSP A - MBP FUSION



54/56

FIGURE 17(ii)

HSP B - MBP FUSION



55/56

FIGURE 17(iii)

MBP

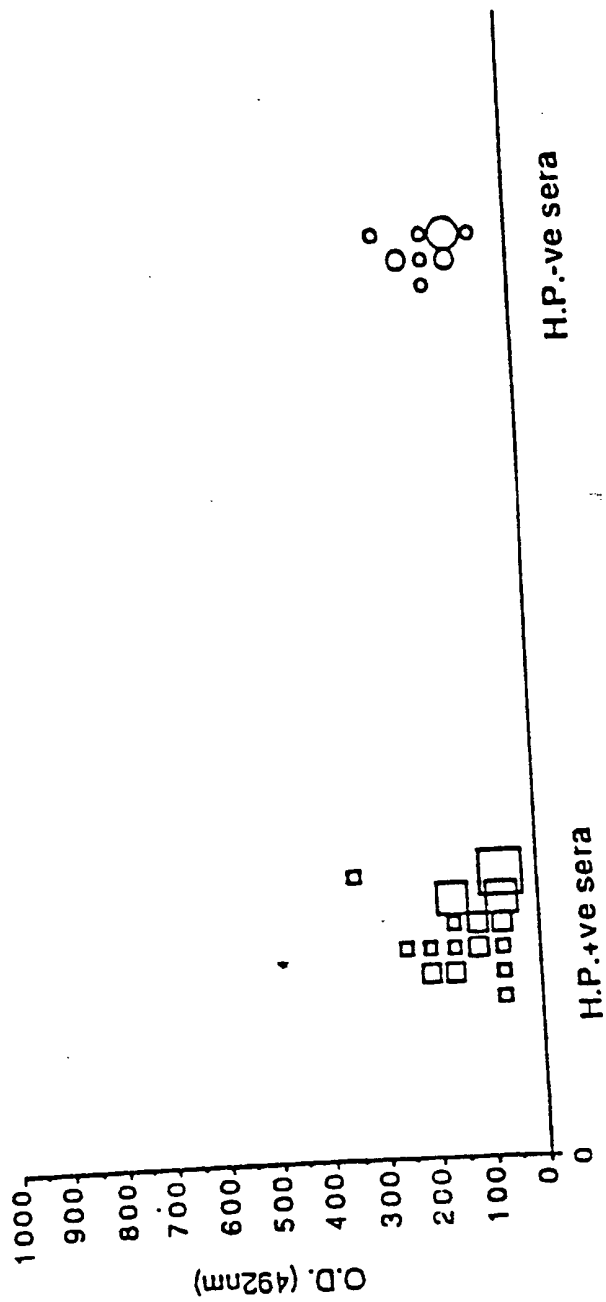
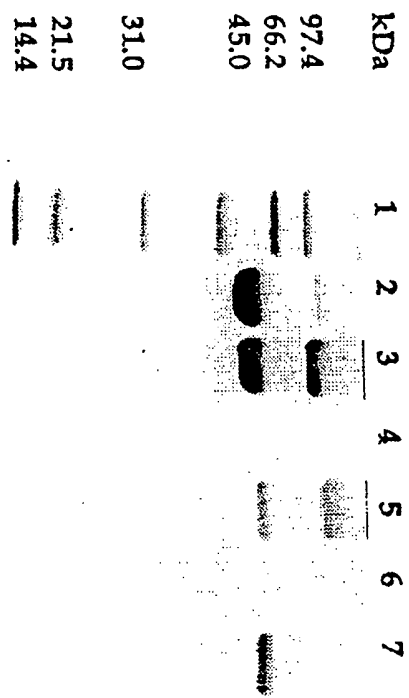


FIGURE 18



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